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Morphological and molecular evidence reveals a new species of *Laetmogone* (Holothuroidea, Elasipodida) from abyssal depths of the south Pacific Ocean

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Abstract

A new elasipodid species of holothuroid, *Laetmogone multiradiolus* **sp. nov.**, was discovered from the abyssal plain adjacent to the Kermadec Trench in the South Pacific Ocean at a depth of 5735 m, representing the deepest record of a *Laetmogone* species. We provide a detailed description of the morphological features of this newly-discovered species and present molecular data for the new species and two congeneric specimens collected from the South China Sea used in the phylogenetic analyses of the family Laetmogonidae. Furthermore, we provide a taxonomic key to *Laetmogone* species and discuss the geographical distribution and species diversity for the family Laetmogonidae. More specimens from broader geographical locations and additional molecular data are needed to better investigate the phylogeny, morphology and biogeography of laetmogonid holothuroids.

Key Words

COI, deep-sea, Pacific Ocean, phylogeny, sea cucumber, taxonomy

Introduction

Holothuroids are prominent members of marine benthic invertebrate communities and they exhibit high diversity in the deep sea (Hansen 1975; Iken et al. 2001; Jamieson et al. 2011). The class Holothuroidea includes the order Elasipodida, which is comprised of four families: Psychropotidae Théel, 1882, Elpidiidae Théel, 1882, Laetmogonidae Ekman, 1926 and Pelagothuriidae Ludwig, 1893. Species in these families are found at different depths (Hansen 1975; Gebruk 1990, 2008; Rogacheva et al. 2013). Laetmogonid holothuroids are restricted to bathyal and abyssal depths, which encompasses both cosmopolitan and endemic species (Reich 2012). *Laetmogone* Théel, 1879, which is one of the six genera within the family Laetmogonidae, was established by Théel (1879), based on specimens collected during the "Challenger" expedition of 1872–1876. Members of this genus are characterised by the presence of papillae along the dorsal radii, the lack of mid-ventral tube feet and circum-oral papillae and the absence of marginal teeth on the wheel ossicles (Hansen 1975). Currently, the genus *Laetmogone* includes 12 valid species (WoRMS 2024).

From October 2022 to March 2023, a joint China-New Zealand scientific expedition conducted an extensive and systematic manned, deep-sea diving survey in the Kermadec Trench in the South Pacific (Peng et al. 2023). During this expedition, we collected more than 110 holothuroid specimens. In this study, we focus on the genus *Laetmogone* in these collections, discuss the geographic distribution and diversity of laetmogonid holothuroids (data on the remaining holothuroid taxa will be published separately) and provide a taxonomic key to *Laetmogone*

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species. After the examination of these specimens, we identified a new species by utilising both morphological features and molecular phylogenetic analyses.

Materials and methods

Sample collection

A single specimen was collected from the abyssal plain adjacent to the Kermadec Trench in the South Pacific Ocean (maximum depth ~ 10,000 m). We used the manned submersible vehicle 'Fendouzhe' at a depth of 5735 m (Fig. 1) as part of the Global Trench Exploration and Diving Program (Global TREnD). The specimen was preserved in 99% high grade absolute ethanol. To perform comprehensive phylogenetic inference on the family Laetmogonidae, we supplemented molecular data from two specimens collected from the South China Sea in the West Pacific Ocean using the manned submersible vehicle 'Shenhaiyongshi' at depths of 3566 m and 3568 m, respectively (Fig. 1) and they were preserved at - 80 °C. The specimen of the new species was registered while we were onboard using the Specify *niwainvert* database of National Institute of Water and Atmospheric Research (NIWA), which was loaned by the NIWA Invertebrate Collection (NIC) to Institute of Deep-sea Science and Engineering (IDSSE). The South China Sea specimens were deposited at IDSSE, Chinese Academy of Sciences (CAS), Sanya, China.

Morphological observation

External morphological features were observed on underwater pictures *in situ* and images taken onboard immediately after collection, which included skin colour, width, length, body shape, the number and arrangement of dorsal papillae, tube feet and tentacles. Some of these features were examined under a dissecting stereomicroscope (OLYMPUS SZX7). A solution of 15% sodium hypochlorite was used to remove the tissues and isolate the ossicles. The general types of ossicles were observed under an optical microscope (OM) and photographs were taken with a scanning electron microscope (Phenom ProX). The ossicles were rinsed with absolute ethanol, dehydrated, bonded on double-sided carbon tapes and coated with gold before SEM observation. We followed Thandar (1998) and Rogacheva et al. (2013) for the terminology of wheel ossicles.

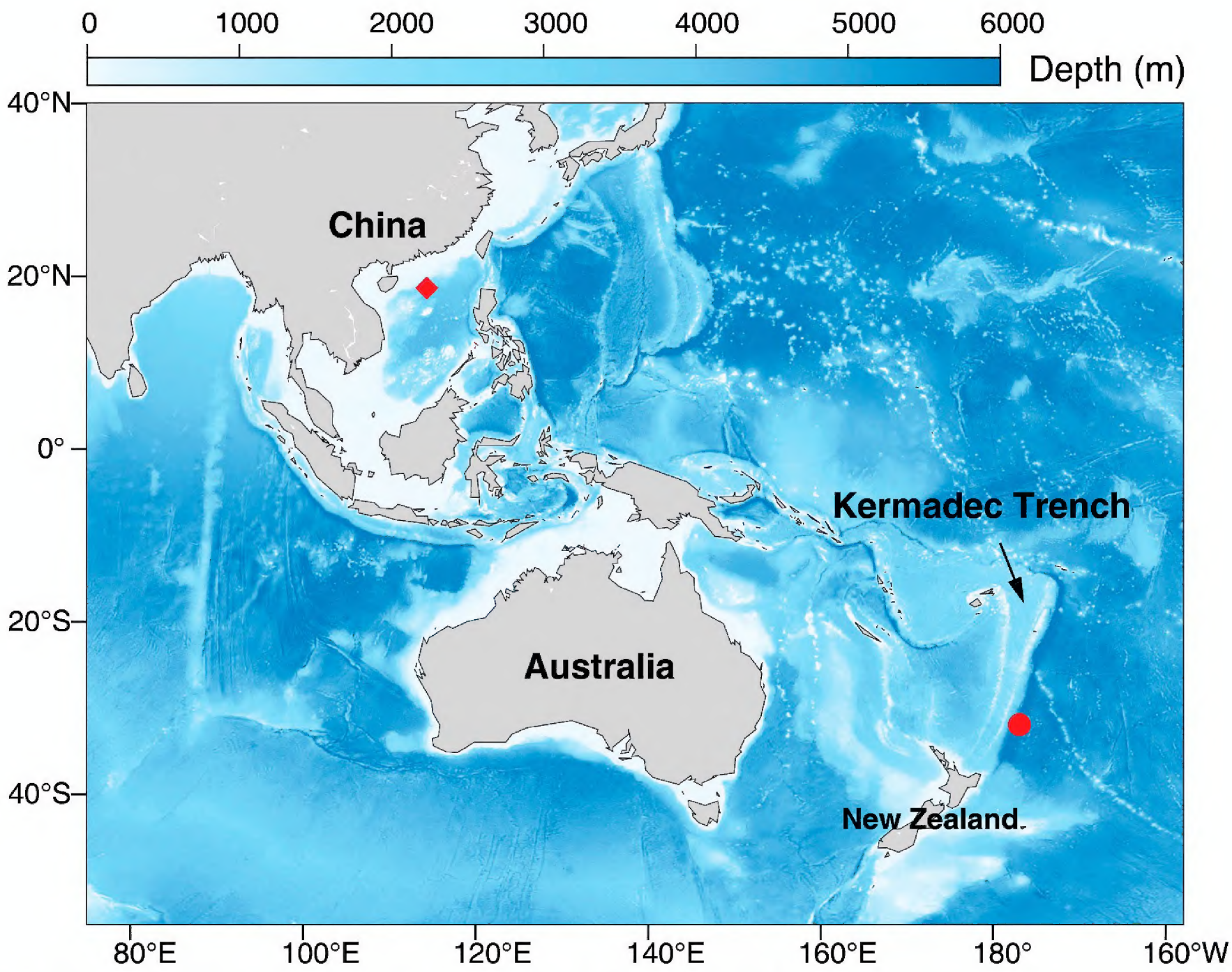

Figure 1. Sampling locations of *Laetmogone multiradiolus* sp. nov. NIWA164015 (red dot) and *Laetmogone* cf. *wyvillethomsoni* Théel, 1879 (red diamond).

DNA extraction and sequencing

For genetic sequencing, we used one specimen of *Laetmogone multiradiolus* sp. nov. collected from the abyssal plain adjacent to the Kermadec Trench and two specimens of *Laetmogone* cf. *wyvillethomsoni* collected from the South China Sea. The genomic DNA of each individual was extracted using a TIANamp Marine Animals DNA Kit (TianGen, Beijing), following the manufacturer's protocol. Mitochondrial cytochrome c oxidase subunit I (COI) was generated for all specimens using the primers outlined in Miller et al. (2017). The PCR conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 40 cycles at 95 °C for 10 s, annealing temperature at 50 °C to 52 °C for 10 s, extension at 72 °C for 10 s and a final extension at 72 °C for 5 min. The 50 µl reaction used for the amplification contained 2 µl of template DNA, 1 µl of each primer and 46 µl of GoldenStar® T6Super PCR Mix (1.1×). PCR products were assessed on GelRed-stained 1.5% agarose gel electrophoresis and sequenced in both directions using the ABI 3730 DNA Analyzer sequencing facility from BGI Genomics, Shenzhen, Guangdong Province, China. The final sequences were deposited in the Science Data Bank of the Chinese Academy of Sciences (Xiao and Zhang 2024) and the GenBank database with the accession numbers PP817260–PP817262.

Barcoarding and phylogenetic analyses

Three COI sequences of three specimens in this study and 23 COI sequences from GenBank, were used for phylogenetic analyses (Suppl. material 1). We used COI sequences of *Peniagone diaphana* (Théel, 1882) and *Peniagone* sp. (Table 1) from the family Elpidiidae as an outgroup. All sequences were aligned using MAFFT7 (Katoh and Standley 2013). Maximum Likelihood (ML) phylogeny was inferred using IQ-TREE with 20,000 ultrafast bootstraps (Minh et al. 2013; Lam-Tung et al. 2015). PartitionFinder2 (Lanfear et al. 2017) was used to choose the best-fitting model, which was GTR + I + G, with all algorithms and AICc criteria. Bayesian Inference (BI) phylogenies were carried out using MrBayes 3.2.6 (Ronquist et al. 2012) under the partition model (two parallel runs, 5,000,000 generations). The first 25% of sampled trees were discarded as burn-in and then the remaining trees were used to construct a 50% majority rule consensus tree. The results were visualised using FigTree v. 1.4.4. (Rambaut 2018). The genetic distances of COI amongst *Laetmogone* species were calculated using MEGA X (Kumar et al. 2018).

Distribution and species diversity of Laetmogonidae

The existing distribution data of Laetmogonid species were obtained from the Ocean Biodiversity Information System (https://obis.org/), the Global Biodiversity Information Facility (https://www.gbif.org/zh/), from published literature and include data from this study. The distribution of six genera in the family Laetmogonidae was illustrated using Generic Mapping Tools, which is a cartographic scripting toolset developed by Wessel and Smith (1995). Using the World Register of Marine Species (WoRMS) and primary literature (Théel 1879; Ludwig, 1893; Mitsukuri 1897; Sluiter, 1901; Fisher 1907; Massin 1987; Thandar 1998; Rogacheva et al. 2013), we obtained data of all accepted species of this family to discuss the geographical distribution of different groups.

Table 1. *Laetmogone multiradiolus* sp. nov. NIWA164015. Number of spokes and central rays and diameter of 100 wheels from body wall, papillae, tube feet and tentacles of the holotype.

Diameter (mm)	Central rays						Spokes							
	4	5	6	8	9	10	11	12	13	14	15	16	17	8–17
0.04	8	–	–	–	–	–	1	2	2	3	–	–	–	8
0.05	2	2	–	–	–	–	1	1	1	–	–	1	–	4
0.06	7	4	–	–	–	–	1	2	2	2	2		2	11
0.07	3	1	–	–	–	–	1	1	1	1	–	–	–	4
0.08	5	4	–	1		1	1	2	3	–	1	–	–	9
0.09	4	4	–		1	1		2	2	1	1	–	–	8
0.10	5	3	1	2	1		1	2	1	1	1	–	–	9
0.12	2	3	–		1	2	1	1	–	–	–	–	–	5
0.13	2	6	–	2	1	1	1	1	1	1	–	–	–	8
0.14	1	4	–	1	1	2	–	–	–	1	–	–	–	5
0.15	–	3	–		1	1	–	–	1	–	–	–	–	3
0.16	1	2	–	1	1	1	–	–	–	–	–	–	–	3
0.17	1	3	–		2	2	–	–	–	–	–	–	–	4
0.18	1	2	–		1	2	–	–	–	–	–	–	–	3
0.19	–	6	–			6	–	–	–	–	–	–	–	6
0.20	2	6	–	1	1	4	1	1	–	–	–	–	–	8
0.21	–	2	–	–	–	–	2	–	–	–	–	–	–	2
0.04-0.21	44	55	1	8	11	23	11	15	14	10	5	1	2	100

Results

Systematics

Order Elasipodida Théel, 1882
Family Laetmogonidae Ekman, 1926

Genus *Laetmogone* Théel, 1882

Diagnosis. "Circum oral papillae absent; mid-ventral tube feet absent; dorsal papillae in single rows, double rows or bands; ventrolateral papillae absent; wheels lacking marginal teeth" [from Hansen 1975: 52].

Type species. *Laetmogone wyvillethomsoni* Théel, 1879.

Other species. *L. billetti* Rogacheva & Gebruk in Rogacheva et al. 2013; *L. biserialis* Fisher, 1907 [syn. *L. neglecta* Mitsukuri, 1912]; *L. fimbriata* (Sluiter, 1901) [syn. *L. parva* Mitsukuri, 1912; *L. selenkai* Mitsukuri, 1912]; *L. ijimai* (Mitsukuri, 1897); *L. interjacens* Sluiter, 1901; *L. maculata* (Théel, 1879) [syn. *L. enisus* Sluiter, 1901]; *L. parvipedata* Massin, 1987; *L. perplexa* Thandar, 1998; *L. scotoeides* (H.L. Clark, 1913); *L. theeli* Ludwig, 1893; *L. violacea* Théel, 1879 [syn. *L. brongniarti* E. Perrier, 1886; *L. jourdainii* Petit, 1885].

Laetmogone multiradiolus sp. nov.

https://zoobank.org/2BC0CDB1-086A-41DE-B71C-A9FD5325D02F

Figs 2–4

Material examined. ***Holotype*** South Pacific • 1 specimen; from the abyssal plain adjacent to the Kermadec Trench; 31°55.54'S, 176°57.09'W; depth 5735 m; 3 Nov 2022; preserved in 99% high grade absolute ethanol; NIWA164015.

Type locality. The abyssal plain adjacent to the Kermadec Trench, the South Pacific, depth 5735 m.

Diagnosis. A member of the genus *Laetmogone* with the following features: Colour uniformly dark violet; tentacles 17, with rounded terminal discs, slightly lobed; calcareous ring absent; papillae conspicuous, nine in each dorsal radius; tube feet 12 pairs, placed in single rows along ventrolateral radii. Body-wall ossicles in form of elasipodid wheels, circular in outline, with 4–5 (rarely 6) central rays, 8–17 spokes, nave covered by a calcareous membrane; rods and few irregular ossicles in papillae, tube feet and tentacles; cross-shaped ossicles absent.

Description. Body long, cylindrical, slightly pointed anteriorly (Fig. 2A, B), 31 cm long, 7.5 cm wide *in situ* (Fig. 2C, D), 11 cm long, 1.5 cm wide in preserved state. Colour uniformly dark violet in both vivo and preserved states (Fig. 2A–F). Tentacles 17, large, 0.6–1 cm in length after several days of fixation, slightly lobed (Fig. 2E, F). Dorsal papillae conspicuous and nine pairs, arranged in single rows along dorsal radii, 0.7–3 cm long after preservation. Ventrolateral tube feet large, robust and conical, up to 12 pairs, 0.4–0.9 cm in a state of preservation. Mouth ventral, anus terminal. Ventrolateral brim absent. Calcareous ring absent; gonads with numerous branched tubules, arranged in several clusters. Only wheel ossicles in dorsal and ventral body walls (Fig. 3A–G), with no sharp distinction between the two types. The nave of every wheel covered by a calcareous membrane (Fig. 3D). Wheels in body wall 0.04–0.21 mm in diameter, central rays 4–5, rarely 6, 8–17 spokes. Rods and wheels in tube feet, papillae and tentacles, wheels similar to those of the body wall. Papillae wheels, rods and few irregular ossicles (Fig. 4A), rods smooth and stout, curved or straight, occasionally possessing a few little spines on rod ends, 0.2–0.5 mm long. Wheels and curved rods in tentacles (Fig. 4B), rods up to 0.8 mm in length, with small spines on both ends, some rods terminally bifurcated (Fig. 4B). Ossicles in tube feet (Fig. 4C) wheels, rods and irregular ossicles, rods similar in size and shape to those in papillae, irregular ossicles may represent a developmental stage of wheels.

More details of the hub, rim, central rays, spoke spaces and orientation of wheels from *L. multiradiolus* sp. nov. were shown in Fig. 5.

We counted the spokes and central rays and the diameter was measured in the specimen collected from the Kermadec Trench (Table 1). A sample of 100 wheels taken from the body wall, papillae, tube feet and tentacles (Table 1) revealed the following data:

A total of 44% of wheels had four central rays, 55% had five and only 1% had six rays. The number of spokes per wheel varied from 8–17 with the majority (84%) having 9–14 spokes (11% with 9, 23% with 10, 11% with 11, 15% with 12, 14% with 13 and 10% with 14 spokes). The largest wheel (0.21 mm) had only 11 spokes. Only wheels with a diameter of 0.06 mm had the maximum spokes (17). A higher spoke number (15–17) is typical of smaller (< 0.1 mm) wheels.

Etymology. The specific name was derived from the Latin words *multi* (many) and *radius* (ray or beam), which refers to the large number of the spokes of wheel ossicles.

Distribution and habitat. Known only from the type locality so far. In this field, the holotype was found on flat sedimentary terrains. Benthic species, no swimming behaviour was observed.

Remarks. *Laetmogone multiradiolus* sp. nov. clearly belongs to the genus *Laetmogone* and possesses 12 pairs of tube feet, which makes *Laetmogone multiradiolus* sp. nov. unique amongst the known *Laetmogone* species. The new species was characterised by a single type of wheel, with 4–5 (rarely 6) central rays and 8–17 spokes. These features place it most similar to *Laetmogone wyvillethomsoni* Théel, 1879. However, *L. multiradiolus* sp. nov. can be differentiated from *L. wyvillethomsoni* by the following features: (1) The number of tentacles and tube feet were different. *L. multiradiolus* sp. nov. had 17 tentacles and 12 tube feet on each side, whereas *L. wyvillethomsoni* had 15 tentacles and at least 15 tube feet on each side. (2) In the new species, larger wheels reached 0.21 mm in diameter, whereas in *L. wyvillethomsoni*, wheels > 0.16 mm in diameter were not found. (3) Wheels in *L. wyvillethomsoni* had 8–14 spokes and some wheels in *L. multiradiolus* sp. nov. had spokes > 14 (8–17).

The new species was also quite different from other *Laetmogone* species. *Laetmogone multiradiolus* sp. nov. differed from *L. maculatus* by the absence of rosettes,

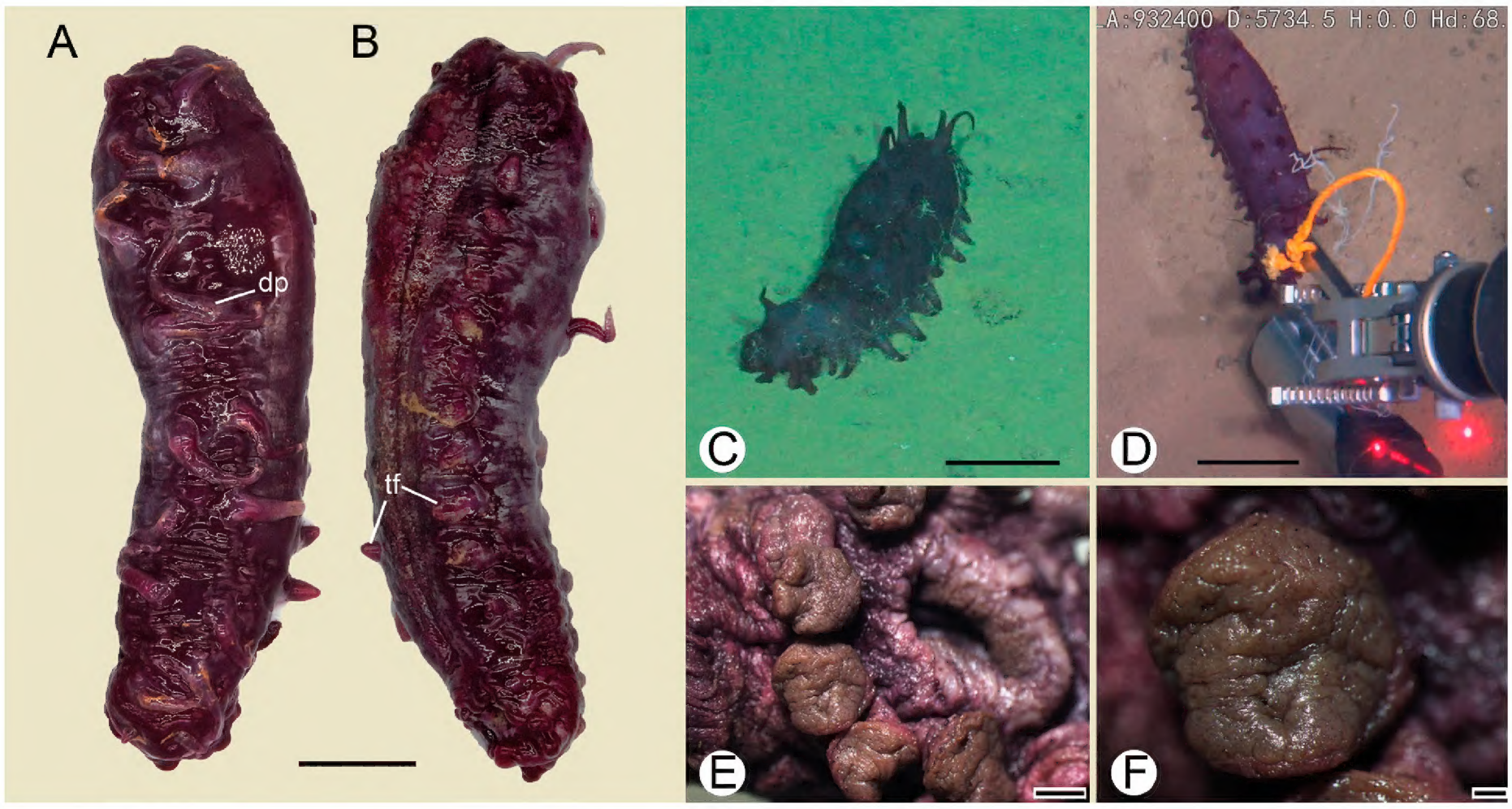

Figure 2. *Laetmogone multiradiolus* sp. nov. NIWA164015. **A, B.** Specimen before fixation; **C, D.** *In situ* images; **E, F.** Tentacles; **dp** – dorsal papillae, **tf** – tube feet. Scale bars: 4 cm (**A–B**); 10 cm (**C–D**); 2 mm (**E**); 500 µm (**F**).

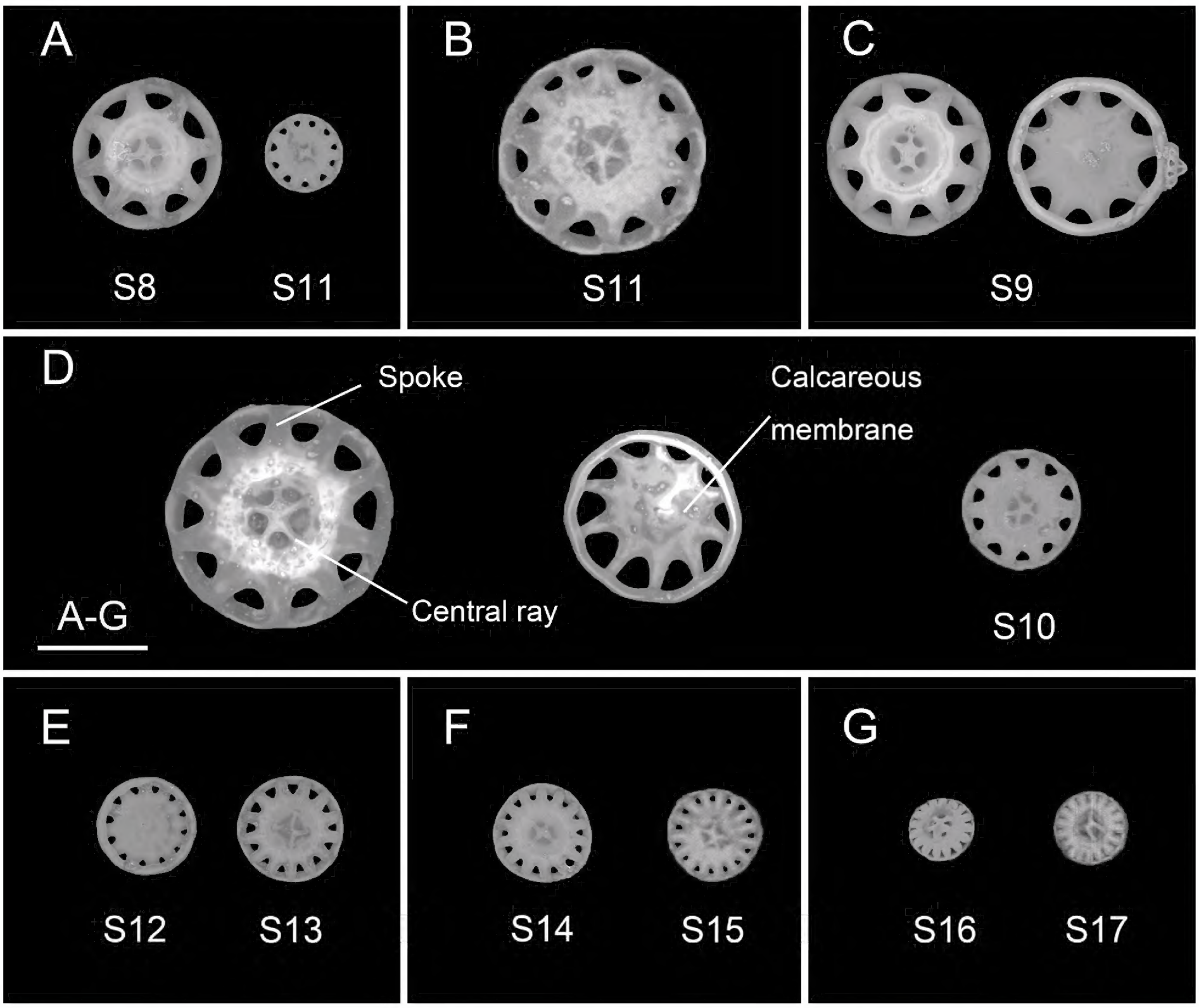

Figure 3. SEM images of ossicles from dorsal and ventral body wall of *Laetmogone multiradiolus* sp. nov. NIWA164015. Scale bar: 100 µm (**A–G**). 'S + number' indicates the number of spokes.

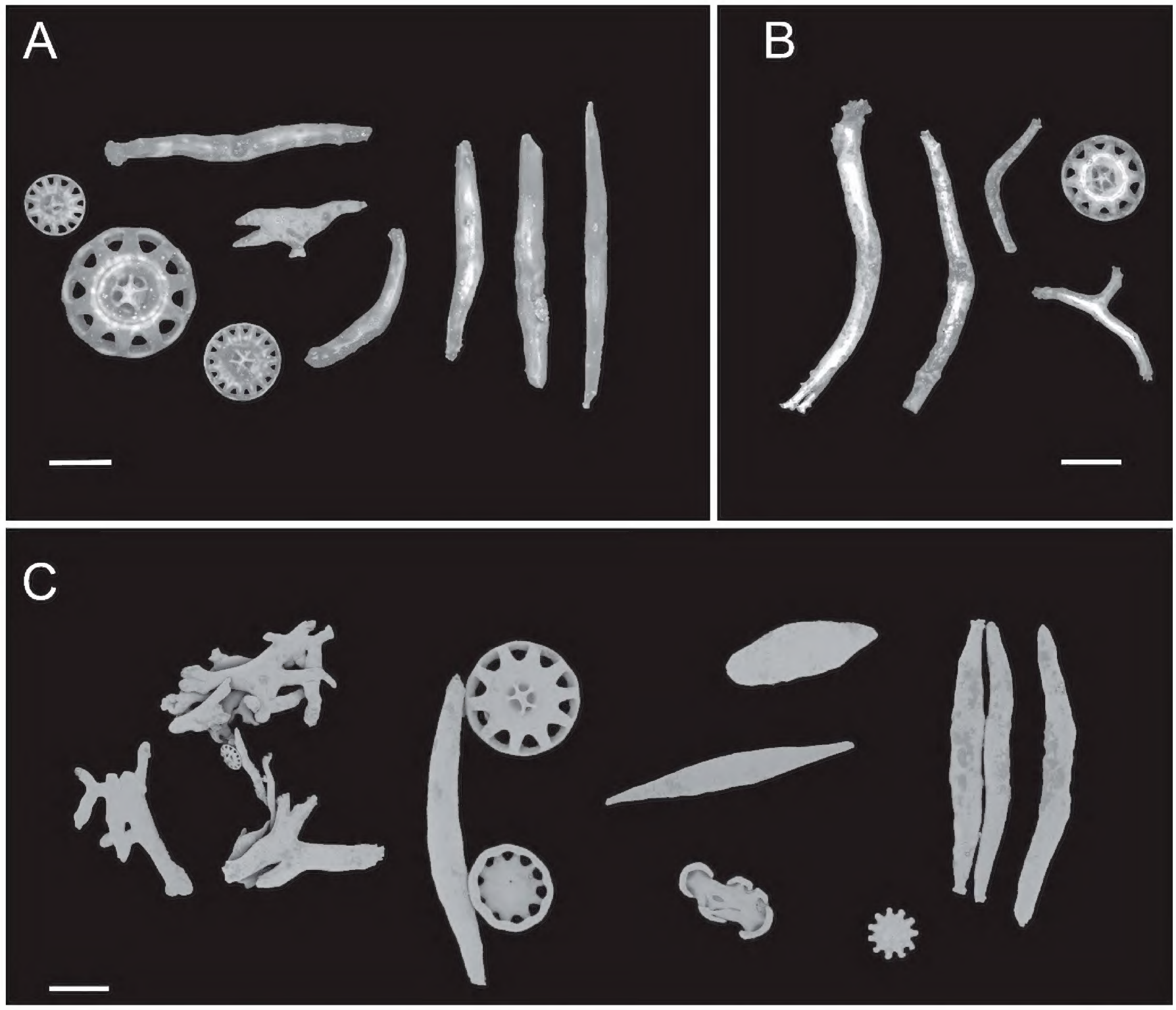

Figure 4. SEM images of dorsal papillae, tentacles and tube feet ossicles from *Laetmogone multiradiolus* sp. nov. NIWA164015. **A.** Papillae; **B.** Tentacles; **C.** Tube feet. Scale bars: 100 μm (**A–C**).

from *L. violacea* Théel, 1879 by the absence of crosses, from *L. scotoeides*, *L. maculata*, *L. fimbriata*, *L. billetti*, *L. ijimai*, *L. biserialis* and *L. pervipedata* by the absence of two distinct types of wheels. The number of tentacles and tube feet makes *L. multiradiolus* sp. nov. and *L. theeli* different; the latter species had numerous tube feet and 20 tentacles, whereas the new species had relatively few tube feet and 17 tentacles. The difference between *L. interjacens*, *L. perplexa* and *L. multiradiolus* sp. nov. is that the new species had large, conspicuous papillae and the papillae of the former two species were small or minute. The dorsal papillae were arranged in two rows along the dorsal radii (four rows along dorsal radii in *L. parvipedata*), which distinguished *L. multiradiolus* sp. nov. from *L. parvipedata*.

Laetmogone cf. *wyvillethomsoni* Théel, 1879

Figs 5, 6

Laetmogone cf. *wyvillethomsoni*, Bribiesca-Contreras et al., 2022: 78–79, fig. 49.

Material examined. West Pacific • 1 specimen; South China Sea; 18°38.20'N, 114°21.29'E; depth 3568 m; 13 July 2019; preserved in - 80 °C; IDSSE-EEB-HS48. • 1 specimen; South China Sea; 18°38.22'N, 114°21.36'E; depth 3566 m; 13 July 2019; preserved in - 80 °C; IDSSE-EEB-HS49.

Description. Body cylinder-shaped and slender. 15.6–24 cm long and 5.2–7 cm wide before preservation (Fig. 6A–E), with the length exceeding the width by more than three times. Mouth anterior, subventral (Fig. 6A–D). Anus terminal, slightly dorsal. Colour dark violet in both vivo and fixed states. Tentacles 15, of similar size. Odd ambulacrum naked. Conical tube feet 21–28, arranged in single rows on ventrolateral radii. Each dorsal radius with a single row of 12–17 long papillae. Ossicle morphology unavailable due to poor condition of the South China Sea specimens.

Remarks. The South China Sea specimens in this study (Fig. 6) belong to the same species (see Table 2 below, 0–0.8% K2P genetic distance) of the Clarion-Clipperton Zone (CCZ) specimen described by Bribiesca-Contreras et al. (2022) and our phylogenetic analyses showed a well-supported clade (see Fig. 7 below, BS = 90, PP = 0.94). The CCZ specimen resembled *L. wyvillethomsoni* closely, as reported by Théel (1879) in the original publication, but no rod-shaped ossicles were

found in the dorsal skin. The South China Sea specimens were in poor condition and mostly fragmented, which made it impossible to obtain ossicles from specific tissue sites. Therefore, we only provided molecular data and images and morphological studies of more specimens are needed to determine the specific taxonomic status of this species. The discovery of specimens collected from the South China Sea expanded their geographical distribution from the eastern to the western Pacific Ocean, with a maximum recorded depth of 3568 m.

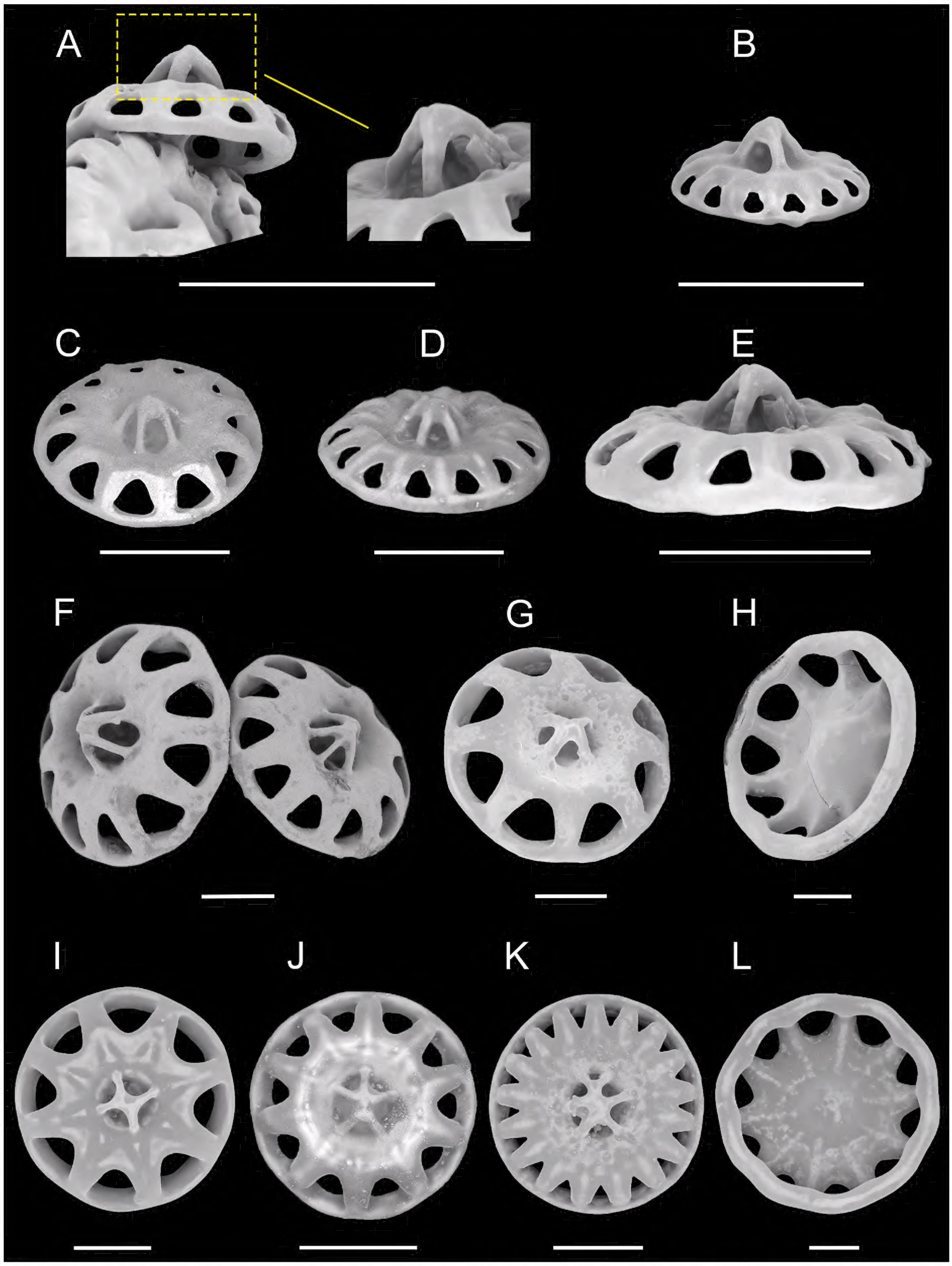

Figure 5. SEM images of wheel-like ossicles from *Laetmogone multiradiolus* sp. nov. NIWA164015. **A–E.** Lower side, lateral oblique view; **F, G.** Lower side, lateral oblique view; **H.** Upper side, lateral oblique view; **I–K.** Lower side (**I.** 4 rays, 8 spokes; **J.** 5 rays, 10 spokes; **K.** 6 rays, 17 spokes); **L.** upper side. Scale bars: 50 μm (**A–L**). The yellow box and line show more details of the central rays.

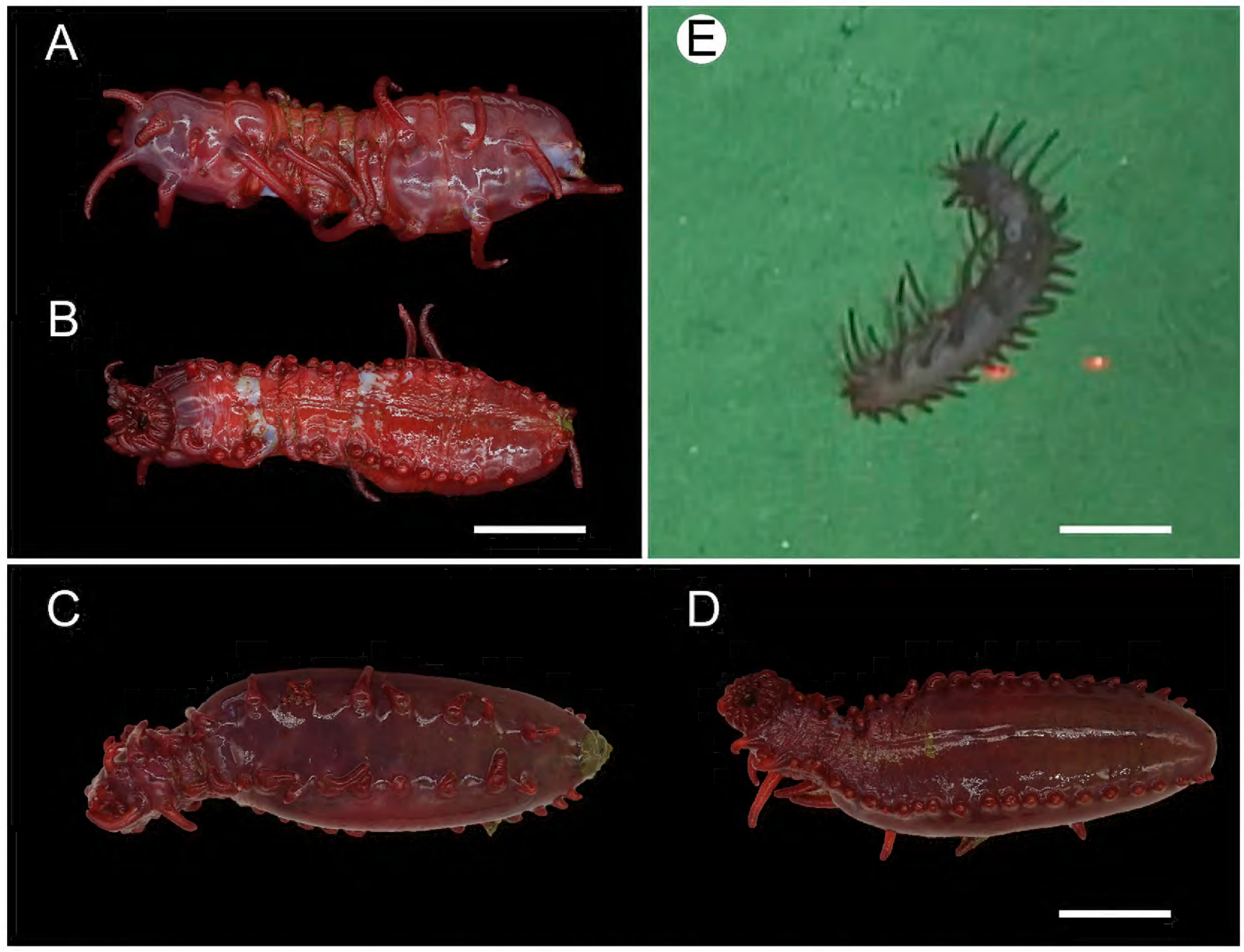

Figure 6. *Laetmogone* cf. *wyvillethomsoni* Théel, 1879 IDSSE-EEB-HS48 and IDSSE-EEB-HS49. **A, B.** Dorsal and ventral view (IDSSE-EEB-HS48); **C, D.** Dorsal and ventral view (IDSSE-EEB-HS49); **E.** *In situ* image. Scale bars: 5 cm (**A–D**); 10 cm (**E**).

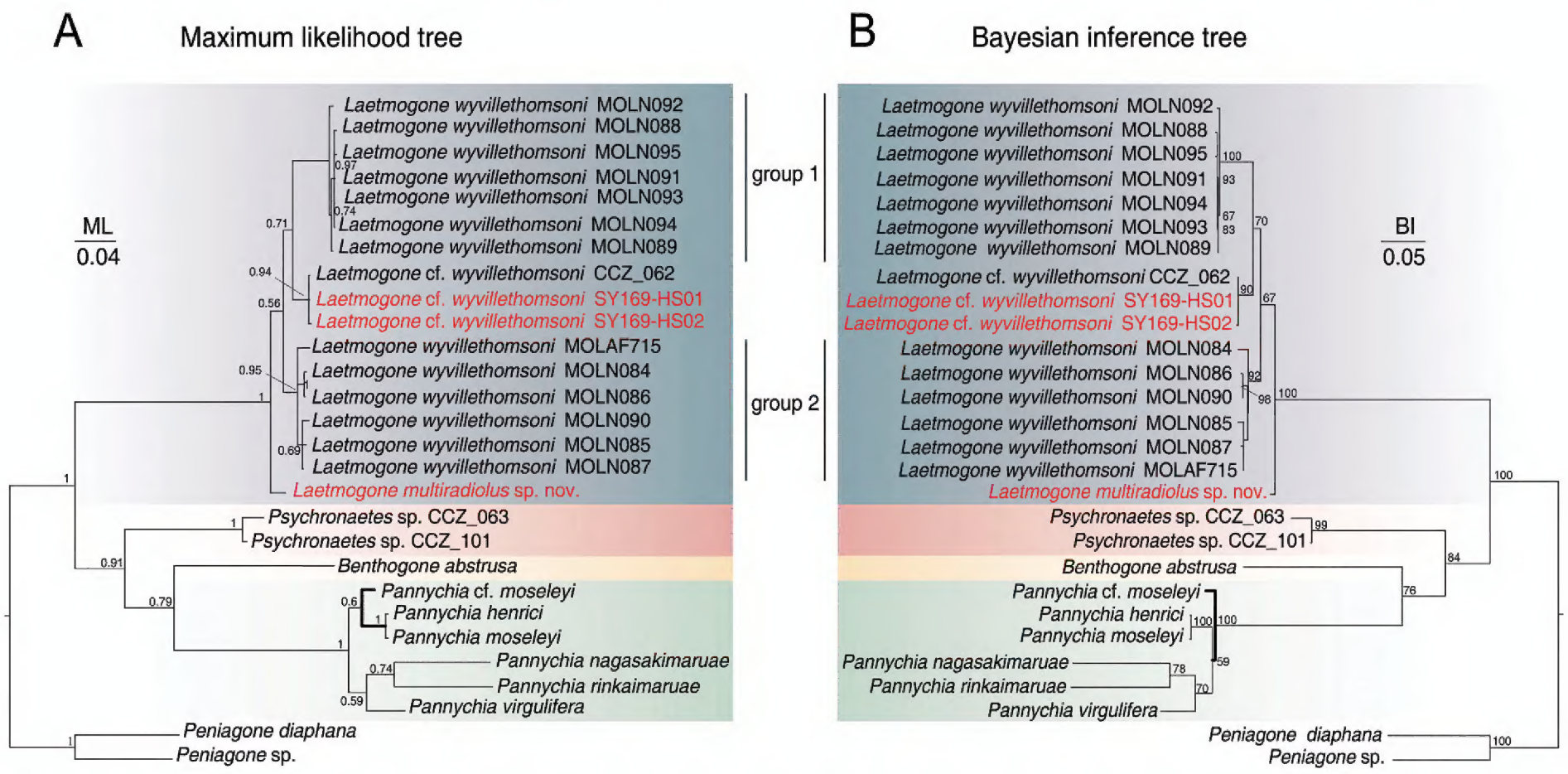

Figure 7. Bayesian Inference (BI) and Maximum Likelihood (ML) phylogenetic analysis, based on COI among species of the family Laetmogonidae. **A.** ML tree, with bootstrap (BS) replications labelled; **B.** BI tree, with posterior probability (PP) labelled. The bold-annotated branches of the ML and BI trees represent the differences in their topologies. Red font: the new sequences provided in this study. BS values < 50 and PP values < 0.5 are not displayed.

Table 2. Estimates of p-distance of the COI gene amongst *Laetmogone* species with available molecular data.

	1	2	3	4
1 *L. wyvillethomsoni* group 1	0%–0.77%			
2 *L. wyvillethomsoni* group 2	5.41%–6.26%	0.15%–2.18%		
3 *L.* cf. *wyvillethomsoni*	4.92%–5.40%	3.38%–4.73%	0%–0.80%	
4 *L. multiradiolus* sp. nov.	5.75%–6.09%	3.39%–4.11%	4.37%–4.48%	-

Intraspecific distance in bold. – means no data.

Key to the species of *Laetmogone* (Adapted from Hansen 1975)

1 Tube feet placed on the edge of a brim which surrounds the whole body. Tentacles 17. Wheels belonging to a single type, with a lower size limit of 0.08 mm; central rays 4 (occasionally 5); spokes 8–12 (15)..... *L. interjacens*
– Brim absent. Tentacles 15. Wheels belonging to one or two types; lower size limit 0.04–0.05 mm 2
2 Papillae conspicuous. Wheels belonging to one or two types 3
– Papillae extremely small. Wheels indistinctly differentiated into two types 9
3 Wheels belonging to two distinct types, one with a central primary cross and rarely exceeding 0.05 mm in diameter, the other with six central rays and always larger than 0.05 mm 4
– Wheels not belonging to two distinct types. Central rays 4–5; spokes 8–18 (20), in the main inversely correlated to the size of the wheels 10
4 Tube feet bulky, narrowing towards the tip 5
– Tube feet crowded, very slender from base to tip, the diameter of the sucking-discs equal to that of the tube feet..... 7
5 Rosette-shaped deposits present ..... *L. maculata*
– Rosette shaped deposits absent 6
6 Tube feet 15 pairs ..... *L. pervipedata*
– Tube feet 22–25 pairs..... *L. ijimai*
7 Large wheels have five central rays ..... *L. billetti*
– Large wheels have six central rays 8
8 Large type of wheel with about nine spokes ..... *L. fimbriata*
– Large type of wheel with about 12 spokes..... *L. biserialis*
9 The larger wheels reaching an upper size limit of 0.3 mm in diameter ..... *L. scotoeides*
– Wheels with a diameter of no more than 0.18 mm..... *L. perplexa*
10 Cross-shaped deposits present ..... *L. violacea*
– Cross-shaped deposits absent 11
11 Tube feet arranged without interspaces, 45–53 pairs ..... *L. theeli*
– Tube feet arranged with interspaces, no more than 33 pairs..... 12
12 Tube feet 15–33 pairs. Tentacles 15..... *L. wyvillethomsoni*
– Tube feet 12 pairs. Tentacles 17 ..... *L. multiradiolus* sp. nov.

Phylogenetic analyses

Phylogenetic analyses of the family Laetmogonidae were performed, based solely on the COI gene (Fig. 7) because sequences of other gene fragments (16S, 12S, H3, 18S, 28S) were insufficient to analyse them. Three COI sequences of the two species (*Laetmogone multiradiolus* sp. nov. and *L.* cf. *wyvillethomsoni*.) were deposited into GenBank (Suppl. material 1). The ML and BI trees were reconstructed to determine the taxonomic status of *L. multiradiolus* sp. nov. using the available COI sequences from the present study and GenBank (Suppl. material 1). In general, the ML and BI tree topologies were similar (Fig. 7), with the exception that the nested relationship between *Pannychia* species was slightly different. The phylogenetic relationships of Laetmogonidae were consistent with the results of the morphological classification. The species in the genera *Laetmogone* (BS = 100, PP = 1), *Pannychia* Théel, 1882 (BS = 100, PP = 1) and *Psychronaetes* Pawson, 1983 (BS = 99, PP = 1) formed monophyletic clades with high support in both the BI and ML trees. *Benthogone abstrusa* (Sluiter, 1901) was supported as a sister taxon to *Pannychia* species (BS = 76, PP = 0.79) and it was the only species in the genus *Benthogone* Koehler, 1895. The *Laetmogone* species were separated into two clades, Clade 1: the new species *Laetmogone multiradiolus* sp. nov. formed an independent clade; Clade 2: *Laetmogone wyvillethomsoni* and *L.* cf. *wyvillethomsoni* formed another clade. Clade 2 was divided into two subclades: (1) *Laetmogone wyvillethomsoni* group 1 clustered with *L.* cf. *wyvillethomsoni* with low support (BS = 70, PP = 0.71); (2) *Laetmogone wyvillethomsoni* group 2 formed another independent subclade.

Discussion

Generic assignment and species delineation

Both morphology and molecular phylogenetic analyses confirmed that the new species belonged to the genus *Laetmogone*. The COI marker has been used frequently to identify many echinoderm species (Hebert et al. 2003; Ward et al. 2008; Layton et al. 2016) and to successfully delimit species of holothuroids (Uthicke et al. 2010; Sonet et al. 2022). In the present study, the genetic distance analysis of COI also indicated its role in delimiting species of *Laetmogone*.

The genetic divergences of the COI gene in *Laetmogone* were calculated using the Kimura 2 parameter (K2P) distance method (Table 2). O'Loughlin et al. (2011) defined species complexes as clades with the same morphospecific identification of their members or with different identifications, but within a 5% K2P distance in COI. In the phylogenetic trees of this study, the

Laetmogone wyvillethomsoni complex from the Ross Sea and Marie Byrd seamounts showed two clades, as reported by O'Loughlin et al. (2011), who distinguished two genetic clades by depth (from 1620–1990 m compared with 2281–3485 m) (Fig. 7). We divided the *Laetmogone wyvillethomsoni* complex into two groups: group 1 and group 2.

The genetic distances amongst specimens in group 1 were very low (0%–0.77%, Table 2) and the distances amongst specimens in group 2 were 0.15%–2.18%. In general, taxonomic units with sequence differences of 2%–5% constituted a 'grey area' where cryptic species may have been present or where specimens from different geographic locations may have represented different species. The taxonomic units with differences < 2% most likely belonged to the same species, but taxonomic units with differences > 5% almost certainly indicated different species (Uthicke et al. 2010). Therefore, all specimens in group 1 belonged to the same species due to the very low genetic distance between them (0%–0.77%, Table 2). The distances between group 1 and group 2 were 5.41%–6.26% (Table 2), which suggested that the *L. wyvillethomsoni* complex (group 1 and group 2) comprised at least two different species. Based on the results of the phylogenetic analyses (Fig. 7) and the genetic distances amongst all the specimens in group 2, which were 0.15%–2.18% (Table 2), we inferred that all the specimens in group 2 belonged to the same species or to several cryptic species. The exact number of species contained in group 2 requires morphological examination and species identification of these specimens. Therefore, the genetic distances between the *Laetmogone wyvillethomsoni* complex were not included in our genetic distance analysis of the genus *Laetmogone*. In our study, the intraspecific distances ranged from 0%–0.8% (Table 2) and the interspecific genetic distances in *Laetmogone* ranged from 3.38%–6.26%.

Laetmogone multiradiolus sp. nov. could be differentiated from other congeners by 12 pairs of tube feet, a single type of wheel, 4–5 central rays and 8–17 spokes. The separations were confirmed by the p-distance analyses, which showed that the genetic distances between *Laetmogone multiradiolus* sp. nov. and other available congeners were 3.39%–6.09%, which was consistent with interspecific genetic distances (3.38%–6.26%) in *Laetmogone*, but higher than the intraspecific variation of *Laetmogone* species (0%–0.8%) in this study. To elucidate the inter- and intraspecific genetic distances in *Laetmogone* species more accurately, it will be necessary to obtain molecular data from more specimens.

Geographic distribution and diversity of laetmogonid species

The family Laetmogonidae is widely distributed in the deep sea. The genus *Laetmogone* is the largest of the six genera, with a total of 13 species, including the newly-described species. *Laetmogone* species are distributed mainly in the Pacific and Atlantic Oceans (Fig. 8A), at depths that range from 130–4410 m (Hansen 1975; Massin 1987; Thandar 1998; Rogacheva et al. 2013). The Pacific Ocean has, by far, the highest diversity of *Laetmogone* species (Fig. 8). Except for *L. perplexa*, which is found only in the Atlantic Ocean (Cape Peninsula), all other species of *Laetmogone* were discovered in the Pacific Ocean (Fig. 8). Three species of *Laetmogone* occur in the South Pacific: *L. fimbriata* (Tasman Sea, New Zealand and Australia, depth 360–705 m), *L. violacea* (Australia and New Zealand, depth 271–1700 m) and *L. wyvillethomsoni* (New Zealand, depth 837–1320 m; the Kermadec Trench, depth 4410 m). The discovery of the new species is the fourth *Laetmogone* species in the South Pacific Ocean and the second *Laetmogone* species found in the Kermadec Trench region. Most *Laetmogone* species are distributed in the lower bathyal to abyssal zone and the only specimen distributed deeper than 3000 m was found in the Kermadec Trench sampling site (Hansen 1975), which was at a depth of 4410 m. The new species, *Laetmogone multiradiolus* sp. nov. was collected from the deepest record for this genus (depth 5735 m) and its discovery broadens our understanding of the distribution of laetmogonid holothuroids at various depths.

Compared with the large number of species contained in the genus *Laetmogone*, there are only six species in *Pannychia* Théel, 1882, three species in *Benthogone* Koehler, 1895 and only one species in each of the following genera: *Apodogaster* Walsh, 1891, *Gebrukothuria* Rogacheva & Cross, 2009 and *Psychronaetes* Pawson, 1983 (Fig. 8). In the genus *Pannychia*, only *Pannychia taylorae* O'Loughlin in O'Loughlin et al. 2013 occurs in the South Indian Ocean, while the other *Pannychia* species are widely distributed in the Pacific Ocean and four species are found only in the North Pacific Ocean (Fig. 8B). In the genus *Benthogone*, *B. fragilis* (Koehler & Vaney, 1905) is found only in the Indian Ocean, *B. rosea* Koehler, 1895 is distributed widely in the North Atlantic, but also in the South Pacific and the Indian Ocean and *B. abstrusa* is distributed in the Indian Ocean and the South Pacific (Fig. 8C). In the other three genera, *Apodogaster alcocki* Walsh, 1891 and *Gebrukothuria profundus* Rogacheva & Cross, 2009 are distributed in the Indian Ocean and *Psychronaetes hanseni* Pawson, 1983 is distributed mainly in the eastern Pacific Ocean (Fig. 8D–F).

Overall, the family Laetmogonidae is very diverse and widely distributed in the Pacific Ocean. Since many areas of the Pacific, including numerous seamounts, have not yet been systematically explored, many more species remain to be discovered. Further investigations should be carried out to provide more additional supporting information (e.g. morphological descriptions, geographic distribution and water depth). In addition, molecular data for the family Laetmogonidae are scarce. Although the genus *Laetmogone* currently contains 13 species, molecular data are currently available for only two species (including the new species described in this study), due

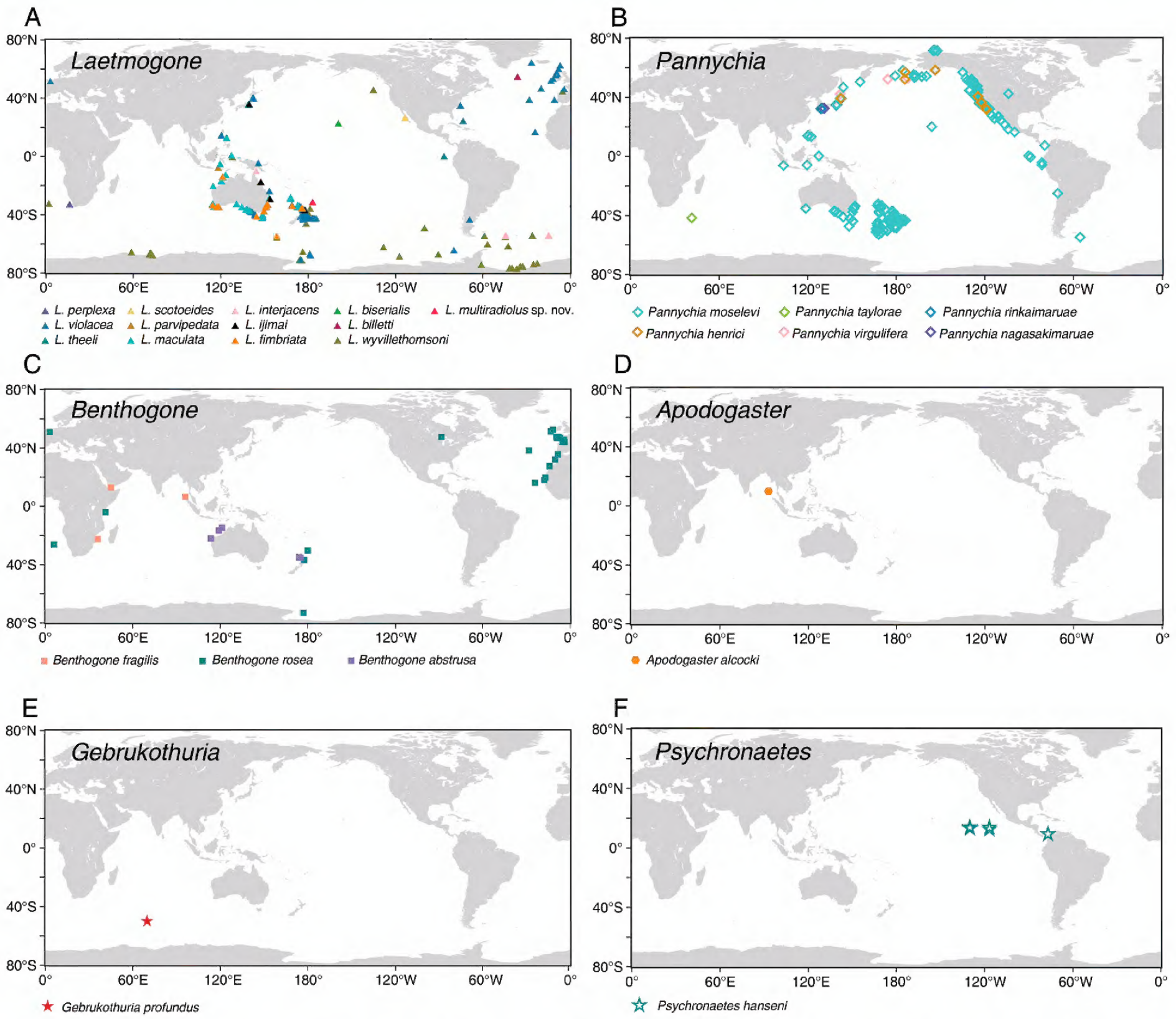

Figure 8. The world distribution of laetmogonid species, based on the Ocean Biodiversity Information System (OBIS) and the Global Biodiversity Information Facility (GBIF) data. **A.** *Laetmogone* Théel, 1879; **B.** *Pannychia* Théel, 1882; **C.** *Benthogone* Koehler, 1895; **D.** *Apodogaster* Walsh, 1891; **E.** *Gebrukothuria* Rogacheva & Cross, 2009; **F.** *Psychronaetes* Pawson, 1983. Species are represented by different colours.

to the fact that around 70% of Laetmogone species were described, based on a single or few specimens collected before 1907. It is necessary to collect more molecular data of Laetmogonidae specimens from different regions and depths to conduct more comprehensive biogeographical analyses and to better understand the relationship amongst laetmogonid species. For this, molecular studies could also incorporate additional molecular markers for species delimitation.

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Competing interests

The authors declare there are no competing interests.

Author contributions

Yunlu Xiao conceived and designed the experiments, performed morphological examination and description, analysed the molecular data, wrote or reviewed drafts of the paper and approved the final draft.

Haibin Zhang conceived and designed the experiments, reviewed and edited drafts of the paper and approved the final draft.

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Supplementary material 1

Species and COI sequences used to reconstruct molecular phylogenetic trees with GenBank numbers and sources

Authors: Yun-Lu Xiao, Haibin Zhang

Data type: docx

Explanation note: GenBank numbers and sources of COI sequences of species in the family Laetmogonidae, to reconstruct molecular phylogenetic trees.

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